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Mianserin Attenuates Naloxone-Precipitated Withdrawal Signs in Rats Acutely or Chronically Dependent upon Morphine[1]

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ABSTRACT

The effects of the atypical antidepressant and serotonin antagonist mianserin on the expression of opiate withdrawal was examined using an acute and a chronic model of morphine dependence. In the first experiment, rats, trained to perform a food-reinforced, autoshaped lever touch response, were injected with naloxone (5 mg/kg) 4 hr after treatment with a single moderate dose of morphine (15 mg/kg). Mianserin (0.25, 1.0 and 2.5 mg/kg) attenuated the naloxone-induced suppression of autoshaped responding. Colonic temperatures were also monitored. Morphine treatment resulted in significant hyperthermia, while precipitation of withdrawal by naloxone produced hypothermia. Mianserin also attenuated the naloxone-induced hypothermia. In the second experiment, rats were implanted s.c. with a single 75-mg morphine or placebo pellet. Withdrawal was precipitated with naloxone (5 mg/kg) 24, 48 and 120 hr post implantation. Mianserin (2.5 mg/kg) blocked or attenuated signs of withdrawal precipitated by naloxone. Naloxone-precipitated weight loss was also attenuated 48 and 120 hr post implantation. At 120 hr post implantation, rats were decapitated 1 hr after the administration of naloxone and trunk blood was collected. Mianserin did not block the naloxone-induced rise in plasma corticosterone levels. Thus, several signs of withdrawal (*e.g.*, behavioral effects, weight loss and hypothermia) seem to involve serotonergic mediation and can be blocked by mianserin, while others (*e.g.*, rise in plasma corticosterone), which may be unaffected by mianserin, may be a reflection of a compensatory response to withdrawal stress, rather than a mediator of maladaptive consequences of withdrawal that are not mediated by serotonin.

Mianserin is a clinically effective tetracyclic antidepressant that does not have the ability to inhibit the reuptake of NE or 5-HT into rat brain tissue or synaptosomes (Leonard, 1974; Raiteri *et al.*, 1976; Goodlet *et al.*, 1977). There is evidence that NE turnover and release are increased by mianserin, which has been interpreted as an antagonistic action at *alpha*-2 adrenoceptors, *i.e.*, a yohimbine-like mechanism (Baumann and Maitre, 1977; Fludder and Leonard, 1979); however, it has been reported (Dwoskin and Sparber, 1983b) that mianserin (1.0–35.0 mg/kg) is unable to block the suppressant effect of a low dose of the *alpha*-2 agonist clonidine on operant behavior, while low doses of yohimbine do antagonize the effect of clonidine. Such results suggest that mianserin was not acting as a yohimbine-like drug in that experiment. Mianserin has also been shown to be a antagonist at 5-HT receptors in peripheral tissue (Vargaftig *et al.*, 1971) and in the central nervous system (Van Riezen, 1972; Maj *et al.*, 1978). Dwoskin and Sparber (1983a) recently demonstrated that behaviorally inactive, low doses of mianserin (0.1–1.0 mg/kg) block the behavioral suppression induced by the 5-HT agonist lysergic acid diethylamide. This effect is most likely due to the high affinity of mianserin for the 5-HT$_2$-binding site (Peroutka and Snyder, 1981), since lysergic acid diethylamide binds with greater affinity (5- to 6-fold) to this presumptive 5-HT$_2$ receptor (deMontigny and Aghajanian, 1977).

Preliminary results in our laboratory (Kleven and Sparber, 1984) indicated that mianserin is effective in reversing the operant behavioral suppression induced by IBMX. Administration of IBMX to opiate naive rats produces a syndrome similar to the morphine withdrawal syndrome precipitated by naloxone (Collier *et al.*, 1974), known as QMWS. Clonidine has been shown to reverse elevations in the major NE metabolite, MHPG (Galloway and Roth, 1983), and block the behavioral signs (Grant and Redmond, 1982) produced by IBMX, as it does the elevations of MHPG (Crawley *et al.*, 1979; Laverty and Roth, 1980) and the behavioral signs (Colelli *et al.*, 1976; Meyer and Sparber, 1976a; Fielding *et al.*, 1978; Sparber and Meyer, 1978) caused by true morphine withdrawal. Therefore, mianserin should also be able to block or attenuate the true morphine withdrawal syndrome as it does QMWS.

ABBREVIATIONS: ANOVA, analysis of variance; HPLC, high pressure liquid chromatography; NE, norepinephrine; 5-HT, serotonin; IBMX, 3-isobutyl-1-methylxanthine; MHPG, 3-methoxy-4-hydroxyphenylethyleneglycol; QMWS, quasi-morphine withdrawal syndrome.

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An acute, moderately low dose of morphine has been shown to induce tolerance and dependence within a few hours (as measured by suppression of operant behavior and increases in plasma corticosterone levels), after administration of naloxone (Sparber *et al.*, 1978; 1979). In the present study, we used the acute dependence model to determine the effect of mianserin on morphine withdrawal in the rat. Along with the suppression of operant behavior, acute dependency upon morphine can also be shown by naloxone-induced decreases in body temperature (Nielsen and Sparber, 1985); therefore, in the first experiment we measured changes in operant responding and body temperature to determine the severity of withdrawal. Because the acute dependence model might not adequately predict the potential efficacy of mianserin in the clinic, we also examined the ability of mianserin to block the signs of withdrawal precipitated by naloxone in rats made chronically dependent upon morphine.

Materials and Methods

Subjects. Male Sprague-Dawley-derived rats, obtained from Holtzman Company (Madison, WI), were individually housed in a temperature (22 ± 1°C)- and humidity (40–50%)-controlled environment under a 12-hr light-dark cycle (on from 0700 hr). Body weights of rats used in the acute withdrawal experiment were gradually reduced to and maintained at 80–85% of the free feeding weight. Rats chronically exposed to morphine were allowed free access to food and water throughout the experiment. The rats were 3 to 4 months of age when the experiment began.

Apparatus. For the autoshaping experiment, testing was carried out in modified operant chambers, each equipped with a retractable lever, a dispenser for the delivery of 45-mg food pellets (BioServ, Inc., Frenchtown, NJ) and a speaker for introduction of white masking noise. The house and cue lights provided a low level of illumination. Exploratory behavior was monitored by measuring contacts with a metal strip 7.5 cm wide and 12.5 cm above the floor on two walls. Contact with the lever in an extended or retracted position (nose-poke response) was also monitored through high resistance drinkometer circuits (<2 MΩ). The operant chambers were enclosed in custom-made sound-attenuated outer chambers equipped with closed-circuit television cameras (Nielsen *et al.*, 1980). **Behavioral sessions were controlled by a custom-made interface using a TRS-80 Color Computer (Tandy Radio Shack, Fort Worth, TX).** Timing and monitoring of behavioral events were provided by a 60-Hz interrupt driven machine language routine. Cumulative records (Ralph Gerbrands, Arlington, MA) were made to allow continuous recording of behavior. Data accumulated by the Color Computer were transferred to cassette tapes at the end of the behavioral sessions for later analyses.

Experimental procedure for acute withdrawal experiment. Autoshaping was accomplished using procedures similar to those previously described (Hughes and Sparber, 1978; Lichtblau and Sparber, 1982). Briefly, rats were placed in operant chambers containing a retractable lever for a 30-min session. A food pellet was delivered every 30 sec, and the lever remained in the retracted position throughout the session. On the second day, the lever was extended every 30 sec and remained extended for 15 sec. From the following session on, lever presentations were made according to a random interval schedule, with the average interval set at 45 sec (minimum, 22 sec; maximum, 68 sec). The lever remained extended for 15 sec or until a lever touch was made, at which time the lever was retracted and a food pellet delivered. Correct responses (extended lever touches resulting in immediate delivery of a food pellet) and the latency to touch the lever during each presentation were recorded. If a correct response was not made, the latency to touch the lever was recorded as 15 sec for purposes of analyses of touch latencies. The rats were exposed to 36 lever presentations per day until all rats were making 35 or 36 correct responses/session (4 days).

Doses of morphine and naloxone were based on previous experiments in which fixed ratio operant responding was completely suppressed 1 hr after morphine, but was not different from control responses 3 to 4 hr later, and in which subsequently administered naloxone suppressed responding (Sparber *et al.*, 1978). The doses of mianserin were based on experiments demonstrating pharmacologic activity at behaviorally inactive doses (Dwoskin and Sparber, 1983a).

Colonic temperature was determined by inserting a lubricated temperature probe (model 702, Yellow Springs Instrument Company, Yellow Springs, OH) 6 cm into the rat's rectum and securing it with a piece of split rubber tubing around the probe and the rat's tail. The rat was then allowed to move freely in a plastic cage, and its temperature was recorded 2 min later from a telethermometer (model 5810, United Systems Corporation, Dayton, OH). The rubber tubing and probe were then removed. Rats were habituated to having their temperatures taken for several days before the experiment. Colonic temperatures were quite stable; the average coefficient of variation for the morning control temperature for the 3 days before drug administration was 0.28% for all rats.

In the first component of the experiment, rats were removed from their home cages in the morning and had their colonic temperatures recorded. They were then injected with mianserin (0.25, 1.0 or 2.5 mg/kg) or water. Thirty minutes later, temperatures were recorded, after which the rats were placed in the operant chambers for a 36-trial autoshaped behavioral session. Immediately after the session, temperatures were again recorded. The rats were randomly assigned to treatment groups (n = 6/group), balanced for weight and average response latency. On the day before drug administration, all rats received water injections to collect baseline data.

The second component of the experiment was run 1 week later with the same rats. Morning temperatures were recorded, and the rats were then exposed to 12 lever presentations. Immediately after the behavioral session, rats were injected with morphine (15 mg/kg) or saline. Temperatures were then recorded 2 and 3.5 hr after the administration of morphine. Mianserin (0.25, 1.0 or 2.5 mg/kg) or water was administered 3.67 hr after morphine and naloxone (5 mg/kg) or saline was administered 20 min later (4 hr post morphine). The rats were exposed to 36 additional lever presentations 10 min after the naloxone injection. Temperatures were recorded 50 min after the administration of naloxone. The experimental protocol is reviewed in table 1. On day 1, all rats were administered vehicle injections to verify the innocuous nature of the injections and to collect baseline data. On day 2, rats were randomly assigned to treatment groups, balanced for weight and average response latency. To determine the effect of withdrawal on responding, the first group (n = 6) was administered morphine, water and naloxone. The effects of various doses of mianserin on acute withdrawal were examined in groups (n = 6/group) that re-

TABLE 1
Experimental protocol

Time	Event
min	
0	Weigh rat and insert temperature probe
2	Record temperature; remove probe; place rat in operant chamber; start behavioral session (12 trials)
10–12	Remove rat from operant chamber; give injection (1 ml/kg b.wt. i.p.); place rat in home cage
132	Insert temperature probe
134	Record temperature; remove probe; place rat in home cage
222	Insert temperature probe
224	Record temperature; remove probe; place rat in home cage.
232	Give injection (1 ml/kg b.wt. i.p.)
252	Give injection (1 ml/kg b.wt. i.p.)
262	Place rat in operant chamber; start behavioral session (36 trials)
292–302	Remove rat from operant chamber
302	Insert temperature probe
304	Record temperature; remove probe

ceived morphine, mianserin (0.25, 1.0 or 2.5 mg/kg) and naloxone.

Experimental procedure for chronic withdrawal experiment. Rats were randomly assigned to be implanted with either a placebo or morphine (75 mg) pellet. Each animal was anesthetized with pentobarbital sodium (30 mg/kg), and a small incision on the back of the neck just posterior to the shoulder blade was made. A morphine (75 mg base, type B) (Meyer and Sparber, 1976b) or placebo (lactose) pellet was inserted under the skin. The incision was then closed with wound clips. Twenty-four hours after implantation, morphine-implanted rats were randomly assigned to receive water-saline, water-naloxone or mianserin-naloxone. Placebo-implanted rats were randomly assigned to receive water-saline or mianserin-naloxone. Mianserin (2.5 mg/kg) or water was injected 30 min before naloxone (5 mg/kg) or saline. This dose of mianserin has no effect on autoshaped behavior (see table 3). Immediately after the naloxone injection, rats were placed in plastic cages and observed for evidence of withdrawal. Each rat was observed for a 15-min period, and the presence of the following signs was noted: diarrhea, rhinorrhea, lacrimation, ptosis, urination, piloerection, teeth chattering, wet-dog shakes, irritability to handling, salivation, ejaculation, attempted escapes and writhings. Animals were also weighed 15, 60, 120 and 180 min after the naloxone injection. At 48 and 120 hr post implantation, placebo- and morphine-implanted rats were randomly reassigned to treatment groups, so as to balance their treatment histories, and withdrawal was precipitated as before. Composite scores were calculated using a method modified from that of Bläsig and co-workers (1973). Those authors found that the various signs of precipitated withdrawal do not all increase with an increasing degree of dependence. Certain signs are more prominant with less dependence and disappear as the degree of dependence increases, while other signs emerge. Signs occurring during mild withdrawal in the study of Bläsig and co-workers were assigned a 1 in our study, whereas those signs they observed during severe withdrawal, indicating greater dependence, were assigned a 3 in our study. Withdrawal signs that they observed during moderate withdrawal were assigned a 2. Table 2 lists the signs and weighting factors used in our study.

TABLE 2
Signs observed during morphine withdrawal and the weighting factor assigned to them

Withdrawal sign	Weighting Factor
Diarrhea	1
Irritability to handling	1
Teeth chattering	2
Writhing	2
Wet-dog shakes	2
Ejaculation	2
Urination	2
Attempted escapes	2
Piloerection	2
Ptosis	2
Rhinorrhea	3
Lacrimation	3

At 120 hr post implantation, rats were decapitated 1 hr after naloxone administration, and trunk blood was collected into heparinized test tubes.

Corticosterone assay. Blood samples were centrifuged for 20 min (250 × *g*; 4°C), and plasma was separated and stored at −70°C until later assayed. The HPLC method used to determine corticosterone levels is a modification of methods of Alvinerie and Toutain (1982) and Matsuzawa and co-workers (1981). The sample mixture contained 0.1 ml of plasma, 100 ng of prednisolone in 0.1 ml methylene chloride as the internal standard, 0.01 ml of 1.25 N NaOH and 1.5 ml of methylene chloride. The mixture was vortexed and then centrifuged at 250 × *g* for 4 min at 4°C; 1.4 ml of the methylene chloride phase were removed and evaporated under a stream of nitrogen. The extract was reconstituted in 0.2 ml of methylene chloride and injected onto a HPLC system (Beckman model 110A pump, a Rheodyne model 7125 injection valve (Rheodyne, Inc., Cotati, CA) and a 100-μl injection loop) equipped with a Waters μ Porasil column (Waters Associates, Milford, MA; 10 μm; 3.0 mm × 30 cm). A 40-mm guard column, repacked frequently with Partisil-10 particles (Whatman, Inc., Clifton, NJ), was placed before the column. Absorbance at 254 nm was monitored with a LDC DuoMonitor (Laboratory Data Control, Inc., Riviera Beach, FL), filtered with a 0.01-Hz cutoff frequency (model 1021A, Spectrum Science Co., Newark, DE). The signal was recorded with an integrator (Hewlett-Packard model 3390A, Palo Alto, CA) and a Servo Graphic recorder (LDC). In the event the integrator did not discriminate a peak, there was an analog record for measurement of peak heights. The mobile phase was prepared by mixing methylene chloride, methanol and glacial acetic acid in an exact ratio of 97:3:0.3. All reagents were HPLC grade and used without further purification. The solvent was degassed by sonication before use. At a flow rate of 2.0 ml/min, retention times for corticosterone and prednisolone were 3 and 10 min, respectively.

Data analyses. For the acute withdrawal experiment, data for extended lever touches (correct responses) and average response latency were analyzed using single-factor ANOVA. Preplanned comparisons of treatment groups with the control group were made using Dunnett's *t* statistic (Winer, 1971). The changes in colonic temperature observed on the day of drug treatment were compared to the changes recorded at the equivalent time on the control day (the day before) using a repeated measures ANOVA, followed by individual comparisons. Pre- and postmianserin temperatures were compared using paired *t* tests.

Data for the composite scores were analyzed using the Kruskal-Wallis H test (Winer, 1971). Preplanned comparisons of treatment groups were made using the Mann-Whitney U statistic (Brown and Hollander, 1977). Plasma corticosterone levels were analyzed using single-factor ANOVA, while naloxone-induced weight loss was analyzed using a repeated measures ANOVA. Individual comparisons were made using the Newman-Kuels test (Winer, 1971).

Comparisons were two-tailed and considered significant if P ≤ 0.05.

Drugs. Morphine SO_4 was purchased from S. B. Penick and Co. (New York, NY), and the morphine pellets were made by the University of Minnesota School of Pharmacy. Mianserin HCl was a gift from Organon International B.V. (Oss, The Netherlands), and naloxone HCl was a gift from Endo Laboratories (Garden City, NY). Pentobarbital sodium was purchased from Abbott Laboratories (North Chicago, IL) in a 50 mg/ml solution. Morphine and naloxone were dissolved in 0.9% NaCl and mianserin in distilled water. Drug doses pertain to the salts. Drugs were administered i.p. in a volume of 1.0 ml/kg. HPLC grade methylene chloride, methanol and glacial acetic acid were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). Corticosterone and prednisolone were purchased from Sigma Chemical Co. (St. Louis, MO).

Results

The first study showed that in the absence of morphine, mianserin (at all doses) had no effect on the number of correct responses made or the average response latency during 36 trials. The spontaneous rise in temperature observed in the control group appeared to be due to the rats being placed in enclosed operant chambers and the frequent handling that occurred during the experiment. The higher doses of mianserin tended to attenuate this rise in temperature or to produce hypothermia (table 3). This hypothermic activity of mianserin may mask its ability to reverse the withdrawal-induced hypothermia.

As seen in table 4, rats administered naloxone 4 hr after morphine made 20 ± 7 (mean ± S.E.) correct responses, with an average response latency of 7.5 ± 2.8 sec. Mianserin (0.25–2.5 mg/kg) attenuated the suppression of responding induced by naloxone in a dose-dependent manner. Using linear regression ANOVAs, significant linear effects for both correct responses and average response latencies were observed, suggesting that the degree of behavioral suppression depended upon the dose of mianserin administered. The highest dose of mianserin (2.5 mg/kg) completely blocked the suppression, in that all rats in this group made 36 correct responses and their average response latency was 0.97 ± 0.20 sec, no different from the control latency of 0.80 ± 0.10 sec.

For the second experiment, the average morning control temperature was 37.4 ± 0.1°C for all rats on both days 1 and 2. Two hours after the administration of morphine, the average colonic temperature was 39.1 ± 0.1°C compared to 37.6 ± 0.1°C at the same time after saline. This hyperthermia was still evident 1.5 hr later. Figure 1 shows that administration of naloxone to rats treated 4 hr earlier with morphine resulted in hypothermia (1.4°C below the average temperature recorded for that group at the same time on day 1 in the absence of morphine). Mianserin at doses of 0.25 and 1.0 mg/kg resulted in colonic temperatures 50 min post naloxone that were not different from the temperatures recorded on day 1 for their respective groups. The effects of mianserin on colonic temperature can best be described as an inverted U-shaped function. The lower doses (0.25 and 1.0 mg/kg) reversed hypothermia, while the highest dose (2.5 mg/kg) had little or no apparent effect upon this parameter, which may have been due to its own hypothermic activity.

Mianserin pretreatment significantly reduced the number of withdrawal signs observed after naloxone administration 48 and 120 hr post implantation (fig. 2), as well as significantly reduced the degree of precipitated weight loss (fig. 3). At 48 hr post implantation, rats undergoing naloxone-precipitated withdrawal lost 9 g within 60 min, while rats pretreated with mianserin lost 5.5 g. When withdrawal was precipitated 3 days later, the withdrawn rats lost 15 g after 60 min, and mianserin-pretreated rats lost 9 g. Mianserin (2.5 mg/kg) was able to completely block rhinorrhea, salivation, wet-dog shakes and ejaculations and to partially block diarrhea, ptosis, urination, lacrimation, piloerection and teeth chattering, while having no obvious effect upon escape attempts and irritability to handling 120 hr post implantation (fig. 4). Writhings were not evident in any group at this time, although they were observed when withdrawal was precipitated 24 and 48 hr post implantation. At 24 hr post implantation, mianserin pretreatment decreased writhings by 50%, and at 48 hr, the presence of writhing was noted in 62.5% of the withdrawn rats and in none of the mianserin-pretreated rats.

The maximal rise in plasma corticosterone levels occurred 60 min after naloxone injection to rats implanted 3 days earlier with a 75-mg morphine pellet (Sparber *et al.*, 1978). For that reason, rats were killed 60 min after precipitation of withdrawal for collection of blood to determine plasma corticosterone levels. Control rats had an average plasma level of 17.9 ± 2.2 μg/100 ml, while rats treated with morphine alone had an average level of 24.0 ± 5.1 μg/100 ml. Placebo-implanted rats, treated

TABLE 3

Effects of various doses of mianserin on autoshaped behavior and colonic temperature

Values represent the mean ± S.E. for six rats in each group. The change in colonic temperature is the change from the morning control temperature taken immediately before the mianserin injection.

Mianserin	Extended Lever Touches (Correct Responses)	Response Latency (15 sec maximum)	Change in Colonic Temperature	
			30 min	60 min
mg/kg				
0	36 ± 0	1.15 ± 0.33	+0.20 ± 0.04**	+0.55 ± 0.11**
0.25	36 ± 0	0.92 ± 0.12	+0.12 ± 0.05	+0.48 ± 0.08**
1.0	36 ± 0	1.07 ± 0.18	−0.32 ± 0.09* †	+0.10 ± 0.09†
2.5	36 ± 0	0.73 ± 0.12	−0.50 ± 0.11** †	−0.15 ± 0.07†

* P < .05, significantly different from the control temperature before drug administration (paired *t* test).

** P < .01, significantly different from the control temperature before drug administration (paired *t* test).

† P < .01, significantly different from the 0 mg/kg group (Dunnett's *t* statistic).

TABLE 4

Mianserin blocks the behavioral suppression precipitated by naloxone in rats acutely dependent on morphine

Values represent the mean ± S.E. for six subjects in each group. The average response latency for all rats ($n = 24$) during the 12 trial control sessions before drug administration was 0.80 ± 0.10 sec.

Mianserin	*Autoshaped Lever Touches*	Response Latency (15 sec maximum)
mg/kg	*no. of correct responses*	
0	20.2 ± 7.0	7.45 ± 2.82
0.25	33.8 ± 1.6*	2.46 ± 0.89
1.0	35.3 ± 0.5*	1.96 ± 0.75*
2.5	36.0 ± 0[a]	0.97 ± 0.20[a]

* $P < .05$, significantly different from the 0 mg/kg group (Dunnett's *t* statistic).

[a] $P < .02$, significantly different from the 0 mg/kg group (Dunnett's *t* statistic).

with mianserin and naloxone, had an average level of 18.4 ± 2.0 μg/100 ml. Morphine-implanted rats undergoing withdrawal had elevated levels, and mianserin pretreatment did not block the rise in plasma corticosterone levels elicted by injection of naloxone (fig. 5).

Discussion

In the first experiment, an autoshaped behavioral test of acute morphine dependence was used to assess the ability of mianserin to alter the severity of morphine withdrawal. Naloxone (5 mg/kg) produced a suppression of behavior and hypothermia in rats treated 4 hr earlier with a single, moderately low dose of morphine. Low doses of mianserin (0.25–2.5 mg/kg) reversed the suppression of autoshaped behavior in a dose-dependent manner. Furthermore, 0.25 and 1.0 mg/kg of mianserin blocked the naloxone-induced hypothermia. In the second experiment, mianserin blocked or attenuated most of the more traditional signs associated with morphine withdrawal and significantly attenuated the degree of naloxone-induced weight loss.

Several biochemical (Fludder and Leonard, 1979), physiological (Engberg and Svensson, 1980) and behavioral (Robson *et al.*, 1978) studies have suggested that mianserin may have antagonistic actions at the *alpha*-2 adrenoceptor. We (Dwoskin *et al.*, 1983) have previously shown that yohimbine, an *alpha*-2 antagonist, exacerbates the behavioral suppression induced by naloxone in acutely morphine-dependent rats performing a fixed ratio operant. Since clonidine, an *alpha*-2 agonist, can block or attenuate opiate withdrawal (*vide supra*), our data are not in agreement with reports of mianserin acting as an antagonist at the *alpha*-2 adrenoceptor. We found the drug to attenuate withdrawal at these doses, rather than potentiate withdrawal in a manner similar to yohimbine.

One of the signs observed in the chronic withdrawal experiment was diarrhea and resultant weight loss. Diarrhea may be due to actions occurring within the central nervous system signalling the gut to respond or to a direct action of morphine and naloxone upon the gut. The guinea-pig ileum was shown to contain opioid receptors that share the properties of opioid receptors in the central nervous system (Kosterlitz and Watt, 1968; Ehrenpreis *et al.*, 1972; Terenius, 1972; Pert and Snyder, 1973). If naloxone is added to a tissue bath containing an ileum chronically exposed to morphine, there is contracture said to be due to a withdrawal response (Ehrenpreis *et al.*, 1972; 1975; Schulz and Herz, 1976). Along with acetylcholine and perhaps other transmitters, 5-HT is involved in this withdrawal-induced contracture (Gintzler, 1979). 5-HT has also been shown to be involved in the actions of morphine in the dog intestine (Burks and Long, 1967; Burks, 1973; Burks and Grubbs, 1974). Furthermore, removal of morphine by abrupt withdrawal or addition of naloxone causes hyperexcitability of the gut associated with 5-HT supersensitivity (Schulz and Goldstein, 1973; Johnson *et al.*, 1978). These studies suggest that 5-HT released during withdrawal could result in a hyperexcitability in the gut which would manifest itself as diarrhea. Mianserin may also be acting directly on the gut to decrease this hyperexcitability and thereby block diarrhea and weight loss.

Although it has been suggested for many years that 5-HT, acetylcholine and/or catecholamines are involved in the phenomena of opiate tolerance and dependence, there has been no entirely satisfactory explanation of their roles in mechanisms underlying these adaptive processes and/or their expression. 5-HT was first suggested to be involved in the development and expression of morphine dependence in the 1960s (Huidobro *et al.*, 1963; Way *et al.*, 1968). The rate of 5-HT turnover in animals rendered tolerant to and dependent on morphine was found to be increased by some investigators (Azmitia *et al.*, 1970; Shen *et al.*, 1970; Maruyama *et al.*, 1971; Herz and Bläsig, 1978), whereas others (Algeri and Costa, 1971; Cheney *et al.*, 1971; Schecter *et al.*, 1972) found no changes. One laboratory claimed that administration of drugs that decrease brain 5-HT levels (*i.e.*, *p*-chlorophenylalanine, a tryptophan hydroxylase inhibitor, and 5,6-dihydroxytryptamine, a 5-HT neurotoxin)

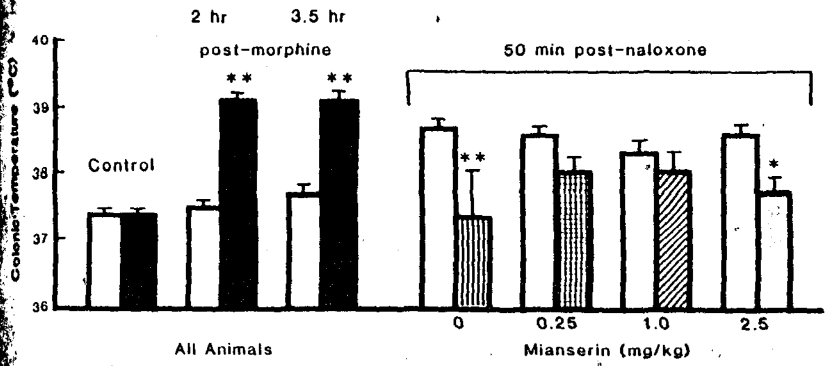

Fig. 1. Mianserin attenuates the hypothermia induced by naloxone in rats pretreated with morphine. Open bars represent the colonic temperature recorded for all rats ($n = 24$) on day 1 when vehicles were injected (control day). Darkened bars illustrate the effect of morphine (15 mg/kg) on the colonic temperature in all rats on day 2, as there were no differences in treatment between groups at these times. Morphine elevated colonic temperature both 2 and 3.5 hr post injection (** $P < .01$ compared with the same time on the control day). Naloxone (5 mg/kg) was administered 4 hr after morphine, and 50 min later resulted in significant hypothermia (** $P < .01$ compared with the same time on control day). Mianserin (0.25–2.5 mg/kg) was administered 20 min before naloxone. With 2.5 mg/kg of mianserin, the change in colonic temperature recorded 50 min post naloxone was significantly different from that at the same time on the control day (* $P < .05$), whereas there were no differences with 0.25 and 1.0 mg/kg.

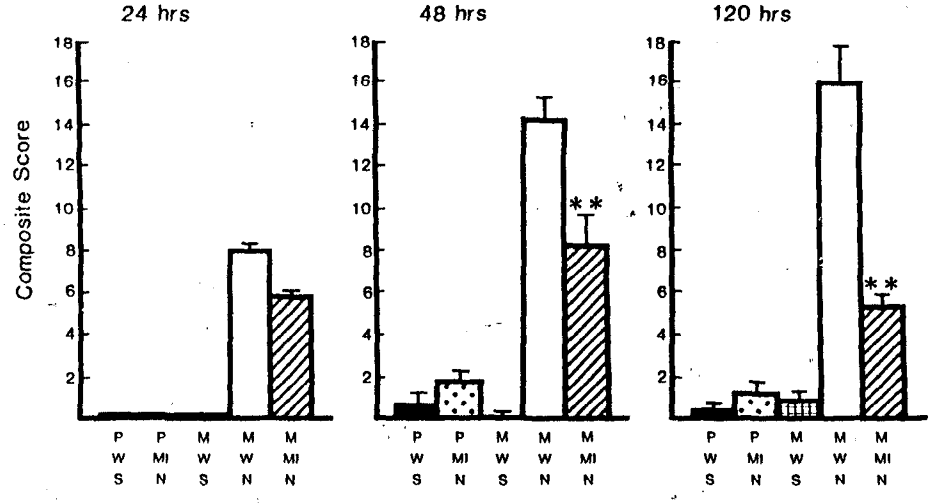

Fig. 2. The effect of mianserin pretreatment on withdrawal signs precipitated by naloxone in rats rendered dependent upon morphine. Data are expressed as composite scores, determined by counting the number of withdrawal signs observed during the 15-min period immediately after the naloxone injection (5 mg/kg) and weighting them according to a scale modified from that designed by Bläsig and co-workers (1973). Each bar represents the mean ± S.E. from seven or eight animals. The first panel illustrates the results when withdrawal was precipitated 24 hr after implantation of a 75-mg morphine (or placebo) pellet; the second panel, 48 hr; and the third panel, 120 hr. The treatment groups are as follows: P-W-S, placebo-water-saline; P-MI-N, placebo-mianserin-naloxone; M-W-S, morphine-water-saline; M-W-N, morphine-water-naloxone; and M-MI-N, morphine-mianserin-naloxone. Significant differences between the M-W-N and M-MI-N group are illustrated (** $P < .01$, by the Mann-Whitney U statistics).

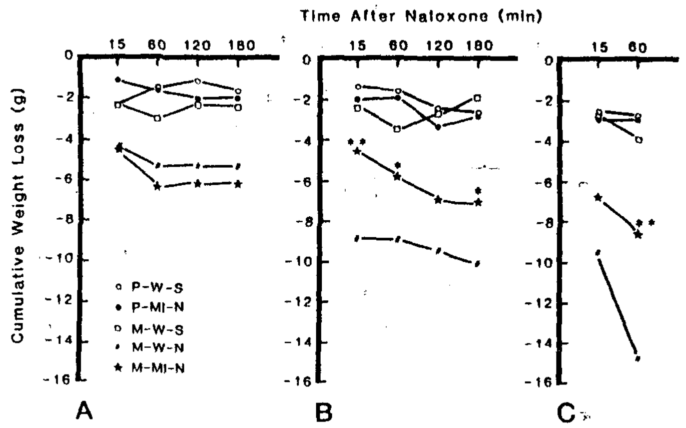

Fig. 3. The effect of mianserin pretreatment on naloxone-precipitated weight loss in morphine-dependent rats. Data are expressed as the cumulative weight loss (grams) at various time points after naloxone administration (5 mg/kg). Rats were implanted with a single morphine (75 mg) or placebo pellet, and naloxone was injected 24 hr (A), 48 hr (B) and 120 hr (C) post implantation. Mianserin (2.5 mg/kg) was administered 30 min before naloxone. Abbreviations for treatment groups are explained in Fig. 2. Significant differences between the M-W-N and the M-MI-N groups are illustrated. (* $P < 0.05$; ** $P < 0.01$, by Newman-Keuls test).

partially inhibits the development of morphine tolerance and dependence (Shen *et al.*, 1970; Ho *et al.*, 1972; 1973), while administration of the 5-HT precursor tryptophan accelerates the rate of dependence development (Ho *et al.*, 1975). Other laboratories (Cheney and Goldstein, 1971; Maruyama *et al.*, 1971; Marshall and Grahame-Smith, 1970) have been unable to confirm these findings. Several studies (Cervo *et al.*, 1981a; 1981b; Romandini *et al.*, 1984) have also attempted to examine the role of 5-HT in the expression of morphine withdrawal, and these results are similarly confusing. Due to the conflicting nature of this literature, the relationship between 5-HT and morphine tolerance and dependence has not been clearly defined. The confusion may be due in part to different populations of 5-HT receptors with opposing actions, inappropriate doses, nonselective drugs used in earlier studies and/or the use of inappropriate methods of studying opiate tolerance/dependence in drug interaction studies (see Sparber *et al.*, 1978; 1979). For example, the traditional signs of withdrawal (*e.g.*, wet-dog shakes, escape attempts) essentially occur only during withdrawal (either true or quasi-morphine withdrawal). A general stimulant or depressant action of the pharmacologic agent may be rendering the animal incapable of exhibiting a particular withdrawal sign, rather than exerting a specific effect upon the underlying process or its expression. Way (1973) has claimed that the conflicting results from laboratory to laboratory and within laboratories may be due to different methods of dependence induction leading to different degrees of dependence development.

Neurophysiological and neuropharmacological studies suggest that there is a catecholaminergic input to the raphe (5-HT) system and a serotonergic input to the noradrenergic neurons of the locus coeruleus (Pujol *et al.*, 1978). During opiate withdrawal, there is an increase in the activity of noradrenergic locus coeruleus neurons, as measured by single-cell recording techniques (Aghajanian, 1978) and alterations in brain levels of MHPG (Crawley *et al.*, 1979; Laverty and Roth, 1980), which can be blocked by clonidine. At low doses, clonidine may stimulate central *alpha*-2 autoreceptors to decrease NE release (Starke and Altmann, 1973) and thereby reduce signs of withdrawal. Clonidine has also been reported to inhibit serotonergic neurotransmission through interactions at *alpha*-2 receptors located on 5-HT neurons (Göthert *et al.*, 1981) and has been found to increase intracellular levels of 5-HT in the dorsal raphe and decrease 5-HT turnover in the striatum (Geyer and Lee, 1984). The elevation in intracellular 5-HT may be due a decrease in 5-HT release. Franz and co-workers (1982) found that clonidine depresses preganglionic sympathetic discharge in the spinal cord and suggest that clonidine may prevent signs of withdrawal by decreasing sympathetic outflow in this manner. There is also evidence that 5-HT neurons exert a facilitatory influence upon central sympathetic outflow (McCall 1984). In that case, mianserin could act as a postsynaptic HT antagonist to decrease this facilitation, thereby decreasing sympathetic outflow. Thus, both clonidine and mianserin could be blocking withdrawal signs by decreasing sympathetic activity, or clonidine could be acting through *alpha*-2 receptors the brain to decrease a serotonergic influence upon sympathetic outflow, either directly on the 5-HT neurons or through catecholaminergic input to the raphe nucleus. Mianserin could also be acting upon a serotonergic influence upon the locus

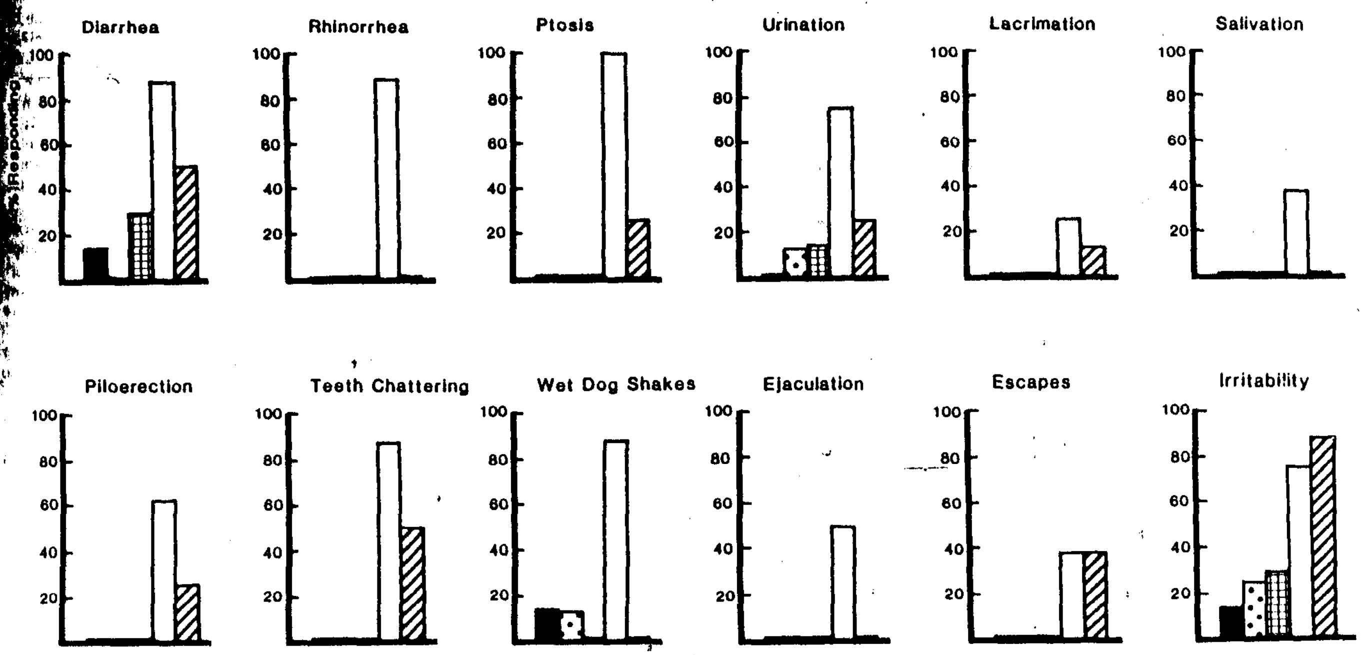

Fig. 4. The effect of mianserin pertreatment on naloxone-precipitated withdrawal signs in morphine-dependent rats. Data are expressed as the percentage of rats ($n = 7$ or 8/group) exhibiting each sign during the 15-min observation period immediately after the naloxone injection (5 mg/kg). Each rat was implanted with a 75-mg morphine (or placebo) pellet 5 days earlier. Mianserin (2.5 mg/kg) was administered 30 min before naloxone. The treatment groups are explained in Fig. 2.

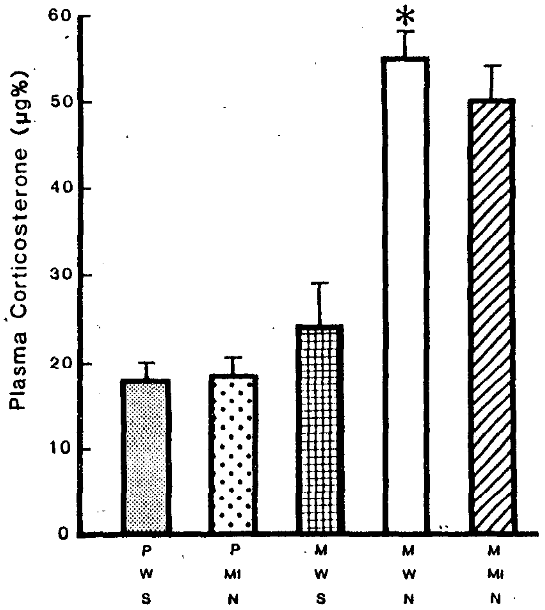

Fig. 5. The lack of effect of mianserin pretreatment on the rise in plasma corticosterone precipitated by naloxone in chronically morphine-dependent rats. Data are expressed as the level of corticosterone (µg/100 ml) in the plasma of rats ($n = 7$ or 8/group) killed 60 min after the naloxone injection (5 mg/kg). The rats were implanted 5 days earlier with a 75-morphine (or placebo) pellet. Mianserin (2.5 mg/kg) was administered 30 min before naloxone. The abbreviations for the treatment groups are explained in Fig. 2. A significant difference between the M-W-N and the M-W-S groups is illustrated (* $P < 0.05$, by Newman-Kuels test).

coeruleus. Further studies are obviously needed to determine the mechanism by which mianserin is acting to attenuate withdrawal.

It has been shown that changes in plasma corticosterone levels are a sensitive and reliable indicator of the severity of opiate withdrawal (Zimmerman *et al.*, 1975; Sparber *et al.*, 1978; Eisenberg and Sparber, 1979). Therefore, one would suppose that agents capable of blocking the other signs of withdrawal should block the rise in plasma corticosterone. However, clonidine, a drug capable of attenuating the withdrawal syndrome in both animals and man, was found to have no effect on the naloxone-induced rise in plasma corticosterone observed after a single dose of morphine (Eisenberg, 1983). It was suggested that clonidine does not affect dependence development, even though clonidine was not coadministered with morphine to test this hypothesis. In our chronic experiment, the rise in plasma corticosterone was also found to be unaffected by mianserin. The rise in plasma corticosterone may be correlated with, but not responsible for, maladaptive behaviors or pathophysiological consequences of withdrawal. Therefore, using plasma corticosterone levels as the only indicator of the severity of withdrawal may not be appropriate when performing drug interaction studies to find agents capable of blocking or attenuating withdrawal "downstream." On the other hand, plasma corticosterone levels may be an extremely sensitive measure of withdrawal that was probably not completely blocked by mianserin, since irritability to handling was also not blocked.

In the present study, mianserin blocked the behavioral suppression and hypothermia induced by naloxone in rats pretreated with a single dose of morphine, along with blocking withdrawal signs in rats implanted with a morphine pellet to induce dependence. Davies and co-workers (1983) examined the effect of mianserin upon chronic dependence using a multiple injection schedule to induce morphine dependence. When mianserin was administered 30 min before naloxone, a low dose of mianserin (1.0 mg/kg) increased the number of wet-dog shakes and writhings, while higher doses (50 mg/kg) significantly attenuated the entire withdrawal syndrome. Therefore, the lower doses of mianserin could have been decreasing the severity of withdrawal, allowing the reemergence of wet-dog shakes and writhings which were masked by the more severe withdrawal signs (*i.e.*, escape attempts and flying) (Bläsig *et al.*, 1973). The doses of mianserin that were effective in attenuating the entire withdrawal syndrome were quite high and may have been acting upon withdrawal through a multiplicity of actions, including a general nonspecific depressant effect upon the central nervous system. However, in our experiment, a much lower dose of mianserin (2.5 mg/kg) was able to block most of the signs of withdrawal. With this dose of mianserin, we are probably observing a more selective effect at 5-HT receptors. Most importantly, the fact that mianserin-like drugs attenuate or block signs of both opiate withdrawal (Neal and Sparber, 1985) and QMWS (Kleven and Sparber, 1984; 1985) indicates that the probable involvement of 5-HT in tolerance and dependence is secondary to the adaptive process and, instead, mediates its expression.

Since mianserin was found to block or attenuate most of the signs associated with morphine withdrawal, such drugs may offer an alternative to the current treatment used in detoxification (*e.g.*, clonidine), which has side effects (*e.g.*, hypotension) that preclude its use in certain patients. The major withdrawal symptom in addicts that is not blocked by clonidine is the difficulty these patients have in falling asleep. One clinical side effect of mianserin is that of sedation, and therefore, mianserin may be able to block the withdrawal symptom upon which clonidine has no effect. Furthermore, although clonidine can reduce the severity of an addict's withdrawal signs and symptoms, the success of detoxification may depend upon his psychological state. If he is depressed or anxious, he may be more affected by withdrawal stresses and less able to resist the urge to resume opiate use (Rounsaville et al., 1985). Depression, which is more common in addicts than in the general population (Robins, 1979; Kleber, 1983), has been found to be importantly related to poorer treatment outcomes (Rounsaville *et al.*, 1982). Because they are effective antidepressants, mianserin-like drugs may offer better alternatives to clonidine-like drugs and increase the chance at a more successful treatment outcome.

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